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STUDIES ON THE BIOCHEMISTRY OF SULPHUR

X. THE CYSTINE CONTENT OF MEAT AND FISH

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STUDIES ON THE BIOCHEMISTRY OF SULPHUR

X. THE CYSTINE CONTENT OF MEAT AND FISH

Some years ago, Sullivan (1926) described a highly distinctive reaction for cysteine, with modifications to include cystine. Later, as detailed in various publications (1929–1930), application was made of the cystine modification to the quantitative estimation of cystine in purified foodstuffs. Certain proteins, such as phaseolin and other beta-globulins, were found to contain small amounts of cystine. Crystalline serum albumin, on the other hand, was found rich in this sulphur-containing amino acid. In verification of the earlier finding by Folin-Marenzi (1929) the cystine content of serum albumin was estimated to be approximately 6 per cent.

In view of the importance of cystine in nutrition, it became of interest to determine the cystine content of natural foodstuffs, meat and fish in particular. Accordingly, investigations were made on the cystine content of round steak, sirloin steak, haddock, halibut, and salmon by determining the cystine in aliquots of the decolorized and neutralized hydrochloric acid hydrolysates of these foodstuffs. The methods employed for cystine estimation were the Okuda (1925, 1929) iodometric method, the Sullivan (1926, 1929) colorimetric method, and the colorimetric methods of Folin and Looney (1922) and of Folin and Marenzi (1929).

Though differing somewhat in experimental results, the various methods mentioned agree in giving the meat and fish a cystine value very much lower than that found for serum albumin. The details of the work are given in the following pages.

EXPERIMENTAL

Freshly cut samples, one-half pound each, of round steak, sirloin steak, haddock, halibut, and salmon, in waxed paper and iced, were obtained from a local market. Aliquots were taken for moisture determination, 10 grams each for hydrolysis of the fresh material, and the remainder in each case was ground up and defatted and dehydrated by treatment with acetone and ether as later detailed.

Determination of moisture.—Moisture determinations were made on the acetone-extracted material by drying to constant weight in an oven at 100°–105° C. Moisture determinations on the fresh material were made similarly and also by drying *in vacuo* at 100° according to the procedure indicated for meat products in the methods of the Association of Official Agricultural Chemists, second

edition (1924). Moisture determined by the official method was slightly lower than that determined by direct heating; but since the amounts were nearly the same, the results were averaged for each foodstuff.

The hydrolysis of fresh material.—The fresh material was hydrolyzed in two ways, namely, (1) in the presence of decolorizing carbon, norite or carboraffin, following a procedure described by Levene and Bass (1928), and (2) by hydrolyzing and then decolorizing—the method usually used.

The first method was used in only a few experiments. It runs as follows: To 10 grams of the fresh foodstuffs were added 10 cubic centimeters of concentrated hydrochloric acid (D.1.18) and norite 2 grams, or, if carboraffin were used, 0.2 gram. The mixture was then placed in a cold crisco bath, which was brought to 120°–125° C. and maintained at this temperature for seven hours. The hydrolysate was filtered on hard paper in a small Buchner funnel. The hydrolysis flask was washed out with 10 cubic centimeters of N hydrochloric acid and the washings were passed through the same Buchner funnel. The clear filtrate in the case of norite and carboraffin (used only once for meats) was brought to pH 3.5 by the addition of 5 N sodium hydroxide added drop-wise with stirring. The solutions made to 100 cubic centimeters with 0.1 N hydrochloric acid were used for cystine studies. The results are given in Tables 1 and 1A.

TABLE 1.—*The cystine content of fresh meat and fish hydrolyzed in the presence of norite*

Material	Moisture	Cystine on moist weight		Cystine on dry weight	
		Sullivan method	Folin-Looney method	Sullivan method	Folin-Looney method
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Round steak	74.91	0.14	0.31	0.56	1.24
Haddock	79.94	.16	.27	.80	1.35
Halibut	75.70	.21	.43	.86	1.77
Salmon	76.78	.20	.38	.86	1.62

TABLE 1A.—*The cystine content of round steak and sirloin steak hydrolyzed in the presence of carboraffin*

Material	Moisture	Cystine on wet weight			Cystine on dry weight		
		Sullivan method	Okuda method	Folin-Marenzi method	Sullivan method	Okuda method	Folin-Marenzi method
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Round steak	75.33	0.133	0.125	0.234	0.54	0.51	0.95
Sirloin steak	74.51	.113	.092	.188	.44	.37	.74

The results obtained by hydrolyzing in the presence of norite or carboraffin indicate that the fish used have a higher cystine content than the meat. This method of hydrolyzing was not continued, since it seemed to offer no advantage over the hydrolysis followed by decolorizing, the customary procedure. In fact, results subsequently given show that in the case of fish, at least, hydrolysis followed by decolorization gives a higher yield of cystine.

The usual procedure in our work with moist foodstuffs was as follows: To approximately 10 grams of each material in small acetylation flasks were added 10 cubic centimeters of concentrated hydrochloric acid. The mixture was then heated for 7 hours in an oil bath kept at 120°–125° C. The hydrolysates were poured into beakers of 100-cubic centimeter capacity and each flask was washed out with 10 cubic centimeters of water. The diluted solution was then decolorized by warming with carboraffin. The meats required 200 milligrams of carboraffin to get a colorless solution; the fish needed only 100 milligrams. The mixtures were filtered and the carboraffin was extracted twice with 5 cubic centimeters of N hydrochloric acid. The combined filtrates were brought to pH 3.5 by means of 5 N sodium hydroxide added dropwise with stirring. The solutions made to 100 cubic centimeters with 0.1 N hydrochloric acid were used for cystine determinations. The methods used were the Okuda iodometric method (1925, 1929), the Folin-Marenzi method (1929), and the Sullivan method as used for casein (1929).

The meat hydrolysates contained only a little cysteine, as judged by the nitroprusside reaction; the fish hydrolysates, on the other hand, contained considerable cysteine. For reasons previously detailed (Sullivan 1929), it is a difficult and somewhat questionable procedure to determine cystine in a mixture of cystine and cysteine. Accordingly, an aliquot of the solution was converted to cystine entirely by passing air through it for several hours until the reaction with sodium nitroprusside and ammonia was negative and no cysteine was demonstrable by the Sullivan reaction. For a satisfactory cystine determination the cysteine present should be converted to cystine in this way.

Meat and fish bought on two separate days considerably apart in time were run for cystine. In the second experiment, hydrolysis was made for both 7 and 20 hours. The difference between the 7 hours' and the 20 hours' hydrolysis is slight in all the methods (within the errors of analysis), and so the findings were averaged. The results on the different samples of the same material differ somewhat, but in general they are of the same order of magnitude.

Since the moist meats and the fish, the salmon in particular, contain other ingredients, fats for example, and since work with these

fresh natural samples also involves a large correction for moisture, it would seem that the findings on the moist samples are not as satisfactory as those from the dehydrated, defatted material subsequently considered in Table 3. Accordingly, instead of the results of the separate determinations on the fresh samples on different days, the average results are given in Table 2.

These results, though not as reliable perhaps as those obtained on the fat-free material given later, should be of value to dietitians interested in the apparent cystine content of the natural foodstuffs as bought for nutrition; for it is a possibility that the reaction between fat or fat-fission products and cystine in acid hydrolysis may obtain in natural digestion—a point as yet uninvestigated.

TABLE 2.—*The cystine content of fresh meat and fish*

Material	Moisture	Cystine on wet weight			Cystine on dry weight		
		Sullivan method	Okuda method	Folin-Marenzi method	Sullivan method	Okuda method	Folin-Marenzi method
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Round steak	74.9	0.132	0.130	0.174	0.522	0.516	0.693
Sirloin steak	75.34	.118	.117	.194	.480	.474	.786
Haddock	80.04	.231	.231	.387	1.16	1.16	1.94
Halibut	78.88	.238	.231	.408	1.13	1.09	1.93
Salmon	72.24	.236	.243	.418	.85	.88	1.50

It may be noted that the results obtained by the Sullivan method and the Okuda method are close together, while the Folin-Marenzi figures are considerably higher. All the methods agree in giving the fresh fish tested a higher cystine value than the fresh meat. For reasons previously touched upon, the data on the natural foodstuffs, containing interfering material, fat especially, are regarded as less reliable than the data obtained from the dehydrated, defatted material presently detailed.

Acetone extracted material.—Some 225 grams of each foodstuff ground in a meat grinder were stirred with 400 cubic centimeters of acetone. After 24 hours at room temperature the acetone was filtered off and the residue was rubbed up in a mortar with 300 cubic centimeters of fresh acetone. After another 24 hours the acetone insoluble material was stirred with 150 cubic centimeters of dry ethyl ether and the mixture was allowed to stand 24 hours. The ether was then filtered off and the residue air dried. In every case the residue was a clean, white, powdery mass.

Hydrolysis of acetone extracted material.—One gram of the dried material was heated with 5 cubic centimeters of 20 per cent hydrochloric acid for 7 hours at 125° C. The contents of the flask were

poured out and the flask washed with 5 cubic centimeters of water. The combined solution was decolorized by adding 50 milligrams of carboraffin and bringing to gentle boiling and filtering. The carboraffin was extracted by further boiling with 5 cubic centimeters of N hydrochloric acid, filtering, and rewashing the carboraffin on the filter with 5 cubic centimeters of cold N acid. The combined acid solutions were brought to pH 3.5 with 5 N sodium hydroxide added dropwise with stirring. The solutions then made to 50 cubic centimeters with 0.1 N hydrochloric acid were used for cystine determinations.

In Table 3 are given data on the foodstuffs dehydrated and defatted by treatment with acetone and ether. Since such purification has been found useful in getting the cystine content of other material the results given in Table 3 are probably nearer the truth than those obtained with the natural foodstuffs given in Tables 1 and 2.

TABLE 3.—*The cystine content of meat and fish powder, on moisture and ash-free basis, (A) 7 hours' hydrolysis, and (B) 20 hours' hydrolysis*

Material	Nitrogen	Moisture	Ash	Cystine on dry ash-free weight					
				Sullivan method, per cent		Okuda method, per cent		Folin-Marenzi method, per cent	
				A	B	A	B	A	B
	Per cent	*Per cent*	*Per cent*						
Round steak	14.36	8.08	3.30	0.73	0.82	0.82	0.87	1.29	1.12
Sirloin steak	14.33	8.08	3.70	.77	.76	.81	.83	1.43	1.17
Haddock	14.41	8.34	4.44	1.11	1.13	1.17	1.15	1.42	1.08
Halibut	14.81	8.18	3.38	1.00	1.18	1.04	1.11	1.13	1.26
Salmon	14.60	8.15	3.76	1.13	1.15	1.11	1.17	1.46	1.87

As shown in Table 3, the cystine values by the Okuda method and the Sullivan method closely approximate, and the findings on the 20 hours' hydrolysate are but little higher than those of the 7 hours' hydrolysate. In general, the values obtained with the Folin-Marenzi method are higher. With the relative agreement between the Sullivan method and the Okuda method, it would seem that the higher results by the Folin-Marenzi method are due to something else than cystine. This conclusion is verified by the following experimental finding: Acting on the statement made by Folin and Looney (1922) that cyanide inhibits the development of color with cystine, 2 cubic centimeters of an aqueous solution of 5 per cent sodium cyanide were added to the cystine standard and to the meat and fish hydrolysates after the addition of sulphite and before the addition of the uric acid reagent. The cyanide treated standard developed practically no color in the Folin-Marenzi procedure, while

the hydrolysates mentioned became blue—a conclusive proof that the hydrolysates contain other material than cystine, reacting positively in the Folin-Marenzi cystine method. The Sullivan and the Okuda methods agree also in giving the fish tested a considerably higher cystine value than the round steak and sirloin steak, which agree fairly closely in cystine content. With both the fresh material and the defatted material the Sullivan and the Okuda methods give a higher cystine content for fish than for meat.

In view of the fact that cystine is an important constituent of foodstuffs, the question as to whether fish has actually a higher cystine content than meat seemed worthy of thorough investigation. This investigation was carried on from several angles, as follows:

(1) The analysis of the protein of meat and fish muscle, freed from water soluble material and from fat.

(2) The analysis of acetone-ether treated meat and fish with the prevention of humin formation.

(3) The glutathione content of meat as compared with fish.

These various points may be taken up separately.

The cystine content of meat and fish protein.—Osborne and Heyl (1908) and Osborne and Jones (1909) prepared ox muscle protein and fish muscle protein by extracting the fresh material with water, alcohol, and ether. These muscle proteins differ from the acetone-ether extracted material described in this paper. In Osborne's procedure any water soluble cystine or cysteine complex (such as glutathione, first isolated by Hopkins (1921)) that might be in fish and meat would be extracted and discarded. Also, what is probably much more important, albumins might well have been extracted in the long contact with water. Since albumins, as tested in this laboratory, have a higher cystine content than globulins, it should be expected that water-extracted proteins would give a lower cystine yield than the acetone-ether extracted material made use of in the work detailed in the present paper. In their early analyses Osborne and Heyl and Osborne and Jones made no cystine determinations. Later, however, on the same type of muscle protein, Jones, Gersdorff, and Moeller (1924, 1925) determined the cystine content of the protein of ox muscle, shrimp muscle, and halibut muscle by means of the Folin-Looney cystine method.

These muscle proteins were obtained from Dr. D. B. Jones of the protein and nutrition division, Bureau of Chemistry and Soils of the United States Department of Agriculture. They were analyzed for cystine by hydrolyzing with 20 per cent hydrochloric acid for 7 and 20 hours, respectively. Since their mode of preparation is radically different, it was not expected that the muscle protein would

necessarily agree with the findings on the acetone-ether dehydrated and defatted material. The results obtained are given in Table 4. In the last column are given the values obtained by Jones and collaborators (1924, 1925) using the Folin-Looney method.

TABLE 4.—*The cystine content of ox and fish muscle protein, (A) 7 hours' hydrolysis, (B) 20 hours' hydrolysis*

Substance	Moisture content	Amount used	Cystine on dry weight						
			Sullivan method		Okuda method		Folin-Marenzi method		Folin-Looney method by Jones
			A	B	A	B	A	B	
	Per cent	*Gram*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Ox muscle	8.02	1	0.92	0.91	0.96	0.90	1.31	1.08	1.55
Shrimp muscle	8.88	1	.83	.89	.90	.88	1.15	1.13	1.78
Halibut muscle	8.65	1	.90	.82	.94	.84	1.27	1.11	1.32

The muscle proteins give somewhat different results from the dehydrated, defatted acetone powders. The ox muscle protein gives a somewhat higher value than the steak powders, and the halibut muscle protein is lower than the acetone-extracted fish material, especially in the Sullivan and the Okuda procedures. In the case of the water-extracted muscle, the ox muscle protein is slightly higher in cystine content than the shrimp and halibut muscles. In the acetone-extracted material, on the other hand, the fish powders give a higher cystine yield than the steaks. The difference between the water-extracted material and the acetone-treated material may lie in the different treatment. Since the acetone treatment would avoid the loss of water-soluble protein, albumins in particular, it seems the preferable procedure and the results obtained seem nearer the truth as regards the relative cystine content of meat and fish as used for nutrition.

The analysis of acetone-ether extracted meat and fish with the prevention of humin formation.—As a rule, when protein is heated with strong acid, a brown black precipitate is formed and the solution is deep brown. These colored bodies have been designated acid-insoluble and acid-soluble humin. Gortner and his collaborators (1915, 1916, 1917, 1923) consider that the amino acid, tryptophane, is the principal origin of the humin resulting from acid hydrolysis of protein. It has been shown also by Grindley and Slater (1915), Gortner (1916), Roxas (1916), and Dowell and Menaul (1919) that other amino acids may be concerned with humin formation. As regards cystine, Roxas found that this amino acid gives

more or less humin when hydrolyzed with acid in the presence of carbohydrates. With Roxas' work in mind, means were sought to prevent humin formation, which seemed to be associated with oxidation phenomena. In previous work on the estimation of cystine in casein and phaseolin (1929), successful attempts were made to inhibit humin formation. Thus, by the use of titanous chloride, as given in Paper IV of this series, Sullivan (1929) found that humin formation could be prevented to a high degree. The cystine findings on casein and phaseolin, however, were about the same order of magnitude with and without the checking of humin. The meat powders gave considerably more humin on hydrolysis than did the fish powders. The difference between meat and fish, as determined on the fresh material, might be attributed to the fact that the meat hydrolysates required more carboraffin to decolorize them than did the fish hydrolysates. The difference between the dehydrated meat and fish, however, could not be laid to differential loss of cystine in decolorizing, since the same amount of carboraffin was used in the fish hydrolysates as in the meat, the meat setting the amount to be used.

To test out the possible effect of humin formation, the meat and fish powders were hydrolyzed in the presence of titanous chloride as follows: 1 gram of the meat and the fish powders, 5 cubic centimeters of 20 per cent hydrochloric acid, and 1 cubic centimeter of 20 per cent titanous chloride ($TiCl_3$) were hydrolyzed for 7 hours in a small acetylation flask in a crisco bath at 125° C. The violet-colored solution (titanous chloride color) was poured into a beaker and the flask was washed out with approximately 7 cubic centimeters of water. Each solution was neutralized by 5 N sodium hydroxide added dropwise with stirring to faint blue with thymol blue—on the average 6 25 cubic centimeters of 5 N NaOH was required. The solution was filtered from the blue titanous precipitate and made to 50 cubic centimeters with 0.1 N hydrochloric acid. All the solutions gave a direct nitroprusside reaction with sodium nitroprusside and ammonia. Twenty-five cubic centimeters were set aside and air was blown through this aliquot until the nitroprusside reaction was negative—that is, until the solution contained only cystine or cystine-like compounds. The oxidized solution was used for cystine determinations by the Sullivan and the Folin-Marenzi methods, the other 25 cubic centimeters were used for determination of cysteine and cystine by the Okuda method (1929), with pure cysteine and cystine, respectively, similarly treated as standards. The results with the meat and fish in the $TiCl_3$ hydrolysis are given in Table 5.

TABLE 5.—*The cystine content of meat and fish, after hydrolysis in the presence of titanous chloride, corrected for moisture and ash in original powders*

Material	Cystine content		
	Sullivan method	Okuda method	Folin-Marenzi method
	Per cent	*Per cent*	*Per cent*
Round steak	0.68	0.77	1.30
Sirloin steak	.88	.86	1.61
Haddock	1.10	1.19	1.27
Halibut	1.07	1.05	1.83
Salmon	1.10	1.11	1.82

As in hydrolysates without titanium as given in Table 3, the results in the titanium procedure, Table 5, with the Okuda method and with the Sullivan method are of the same order of magnitude, while those with the Folin-Marenzi method are higher. The results with the two methods which are in relative agreement indicate little if any increase, in general, in cystine values by the use of titanous chloride to stop humin formation. It would seem that little if any cystine was lost in humin formation in the ordinary hydrolysis. The Folin-Marenzi method, however, gives in general a higher value for cystine in the titanium treatment as compared to the normal hydrolysis. The increase does not lie in real cystine values, since the other methods do not show it but can be attributed to the presence of non-sulphur compounds of high-reducing capacity.

Looking at the question judicially it would seem a justifiable conclusion that the formation of humin in the acid hydrolysis makes little difference in the cystine content of the hydrolysate. The definite conclusion seems deductible also, that the fish worked with have a higher cystine content than the meat.

At this point it would seem proper to refer to the work of others on the cystine content of meat and fish. In addition to the work of Jones and collaborators (1924, 1925) on fish muscle protein previously mentioned, Ogura and Fujikawa (1925) determined the cystine content of various muscle proteins by Okuda's iodine method—among them the muscle protein of several fish. Okuda (1925) lists the cystine content of a number of fish. The average cystine in both cases is slightly less than 1 per cent. Teruuchi and Okabe (1928), using a modified Okuda method, found the cystine content of defatted and desiccated beef and salmon to be 0.66 per cent and 0.58 per cent, respectively. The latter results are lower than those found in this laboratory for beef and salmon dehydrated and defatted by acetone. The lower results by Teruuchi and Okabe, especially in the case of salmon, may be due to the fact that they desiccated by heat—a pro-

cedure which tends to give low cystine results. Recently, Okuda (1929) gave the total cystine (cysteine plus cystine) of various proteins precipitated by sulphosalicylic acid, such as hen muscle protein 0.82 per cent; carp 0.94 per cent; eel 0.96 per cent; lobster 0.96 per cent. Ingvaldsen (1929) studied the cystine content of fish meal, made by cooking in water at 115°–120° C. for one hour, pressing the coagulum, and drying. He gives the cystine content determined by the Folin-Looney method as follows: Fresh cod meal dried *in vacuo* at 95° C. 0.98 per cent; storage salmon 0.76 per cent; herring 0.74 per cent. Since the workers listed used different procedures—heating, drying at high temperatures, or precipitation with sulphosalicylic acid—their results can not well be compared with the results in the present paper, in which the unfavorable effects of water extraction, of heating, etc., were avoided. It may be noted, however, that Okuda's results gave the fish muscle protein a higher cystine value than hen muscle protein.

The glutathione content of meat and fish.—Glutathione, now known from the work of Hopkins (1929) and Kendall (1929) to be a tripeptide composed of cysteine, glutamic acid, and glycine, was early shown by Hopkins (1921) to be the major part of the sulphydril compounds which can be readily extracted from tissues. The evidence to date as to its function in tissue as given by Meldrum and Dixon (1930) seems to be that glutathione in combination with a small amount of cysteine, formed at need or actually present, plays an important rôle in cellular respiration, oxidation, and reduction. Since the fish tested showed a higher cystine content than the meats, as judged by the hydrolysate, it became of interest to determine whether the glutathione content of fish was greater than that of meat.

In his study of the sulphydril content of tissues, Okuda (1929) found sulphosalicylic acid a very satisfactory protein precipitant. Accordingly, 15 grams each of freshly bought round steak, sirloin steak, haddock, halibut, and salmon were cut into strips and ground with 20 cubic centimeters of N sulphosalicylic acid and sand. The solution was filtered off by suction on a Buchner funnel. The residue was scraped off the filter and reground with 20 cubic centimeters of N sulphosalicylic acid. The combined filtrates were made to 50 cubic centimeters with 0.1 N hydrochloric acid. The tests by the Sullivan reagent, which reacts only with cysteine or cystine reduced by sodium cyanide, were negative. With a negative Sullivan reaction the Okuda (1929) cystine method can be used as a measure of glutathione or similar thio compounds.

Under such conditions we have found the Okuda procedure a satisfactory measure of the readily extracted disulphide and sul-

phydril compounds in tissue. Accordingly, each solution was tested by the Okuda method with and without reduction to give total disulphide and the reduced, or sulphydril, compounds. Since the Sullivan method and the Okuda agree on hydrolysates of the fresh material it is highly probable that the (S–S) or (S–H) compounds found in the sulphosalicylic acid extract are oxidized and reduced glutathione. The standards used were cysteine and cystine. With proper computations the glutathione content of the various foodstuffs is as given in Table 6.

TABLE 6.—*The glutathione content of fresh meat and fish*

Material	Reduced glutathione	Total glutathione
	Per cent	*Per cent*
Round steak	0.028	0.033
Sirloin steak	.031	.036
Haddock	.018	.019
Halibut	.013	.013
Salmon	.013	.013

The meat showed a higher extractable glutathione content than the fish. The cystine equivalent of the total glutathione found is but a small part of the cystine found in hydrolysates of meat and fish.

SUMMARY

The cystine content of round steak, sirloin steak, haddock, halibut, and salmon was determined by estimating cystine in hydrolysates of the fresh material and of tissue powders dehydrated and defatted by acetone and ether.

The methods employed were the Sullivan, the Okuda, and the Folin-Marenzi cystine methods.

The Sullivan colorimetric method, and the Okuda iodometric method gave results of the same order of magnitude. The Folin-Marenzi results on the same hydrolysates were higher.

The acetone-ether extracted material seems best suited for cystine determinations. The Sullivan and the Okuda methods give fish a higher cystine content than meat. The Folin-Marenzi method indicates no great difference between fish and meat.

Sodium cyanide added before the addition of the uric acid reagent stops color formation by cystine but only partially checks color formation by the meat and fish hydrolysates.

The Folin-Marenzi cystine method gives a positive reaction with some noncystine compound in the hydrolysates.

The evidence at hand indicates that the cystine findings for meat and fish by the Sullivan and the Okuda methods are the more reliable.

By these two methods the average cystine content of meat is about 0.8 per cent on the moisture-ash-free basis, and by the three, that of fish is about 1.2 per cent.

By using the cystine values obtained on the acteone extracted material and correcting for the moisture of the fresh material, the cystine content of meat as bought is approximately 0.19 per cent, of fish as bought 0.26 per cent.

The prevention of humin formation by means of $TiCl_3$ gives no higher cystine values.

The glutathione content of meat was found slightly higher than that of fish. In both cases the cystine represented by glutathione is but a small part of the total cystine.

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